Pharmacological Characterization of 1-Aminoindan-1,5-dicarboxylic Acid, a Potent mGluR1 Antagonist

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ABSTRACT

We examined the pharmacological profile of 1-aminoindan-1,5-dicarboxylic acid (AIDA), a rigid (carboxyphenyl)glycine derivative acting on metabotropic glutamate receptors (mGluRs). In cells transfected with mGluR1a, AIDA competitively antagonized the stimulatory responses of glutamate and (1S,3R)-1-aminoindan-1,3-dicarboxylic acid [(1S,3R)-ACPD] on phosphoinositide hydrolysis (pA2 = 4.21). In cells transfected with mGluR5a, AIDA displayed a much weaker antagonist effect. In transfected cells expressing mGluR2, AIDA (≤1 mM) did not affect the inhibition of forskolin-stimulated adenylate cyclase activity induced by (1S,3R)-ACPD, but at large concentrations, it displayed a modest agonist activity. In rat hippocampal or striatal slices, AIDA (0.1–1 mM) reduced the effects of (1S,3R)-ACPD on phospholipase C but not on adenylate cyclase responses, whereas (+)-α-methyl-4-carboxyphenylglycine (0.3–1 mM) was an antagonist on both transduction systems. In addition, AIDA (0.3–1 mM) had no effect on mGluRs coupled to phospholipase D, whereas (+)-α-methyl-4-carboxyphenylglycine (0.5–1 mM) acted as an agonist with low intrinsic activity. In rat cortical slices, AIDA antagonized the stimulatory (mGluR1-mediated) effect of (1S,3R)-ACPD on the depolarization-induced outflow of d-[3H]aspartate, disclosing an inhibitory effect ascribable to (1S,3R)-ACPD activating mGluR2 and/or mGluR4. Finally, mice treated with AIDA (0.1–10 nmol i.c.v.) had an increased pain threshold and difficulties in initiating a normal ambulatory behavior. Taken together, these data suggest that AIDA is a potent, selective and competitive mGluR1a antagonist.

The relatively large number of agonists, antagonists and modulators that are available with high affinity and remarkable selectivity for ionotropic glutamate receptors have significantly contributed to the advancement of our knowledge on the physiology and physiopathology of glutamate-mediated neurotransmission (Collingridge and Lester, 1989; Lodge and Collingridge, 1990; Watkins et al., 1990). Glutamate interacts not only with ionotropic receptors but also with G protein-linked receptors, or metabotropic glutamate receptors; PET, phosphatidylethanol; PLC, phospholipase C; PLD, phospholipase D; i.c.v., intracerebroventricular; s.c., subcutaneous; i.p., intraperitoneal.

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ABBREVIATIONS: (1S,3R)-ACPD, (1S,3R)-1-aminoindan-1,5-dicarboxylic acid; AIDA, (RS)-1-aminoindan-1,5-dicarboxylic acid; BHK, baby hamster kidney; (S)-4C-PG, (S)-4-carboxyphenylglycine; IP, inositol phosphate; (+)-MCPG, (+)-α-methyl-4-carboxyphenylglycine; mGluR, metabotropic glutamate receptor; PET, phosphatidylethanol; PLC, phospholipase C; PLD, phospholipase D; i.c.v., intracerebroventricular; s.c., subcutaneous; i.p., intraperitoneal.

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been introduced, such as the development by two independent groups of transgenic mice lacking mGluR1 (Aiba et al., 1994a, 1994b; Conquet et al., 1994). Despite the potential usefulness of these types of models, conflicting data have been obtained in these laboratories as a possible result of compensatory events that may occur in mGluR knock-out animals during development. Therefore, the availability of selective agonists and antagonists still needs to be viewed as an important strategy, not only for possible therapeutic applications but also to understand the functional role of mGluRs.

(Carboxyphenyl)glycines represent the most interesting class of mGluR antagonists characterized so far, but unfortunately none of them are selective. In particular, (+)-MCPG is able to antagonize both mGluR1 and mGluR2, (S)-4-(1-carboxy-3-hydroxyphenyl)glycine is an antagonist of mGluR1 but an agonist of mGluR2 and (S)-4-carboxy-3-hydroxyphenylglycine is an antagonist of mGluR1 and an agonist of mGluR2 and mGluR5 (Brabet et al., 1995; Cavanni et al., 1994; Hayashi et al., 1994; Kingston et al., 1995; Roberts, 1995; Thomsen et al., 1994a; Watkins and Collingridge, 1994). In the search for more selective agents, we recently reported that AIDA, a 1-aminoindanedi-carboxylate in which the (carboxyphenyl)glycine moiety is inserted in a constrained framework, is an antagonist of mGluRs.

Measurements of second messengers. The assay conditions for measurements of PLC-catalyzed [3H]IP formation in transfected cells expressing mGluRα (Thomsen et al., 1993, 1994a) or in brain slices (Lombardi et al., 1993, 1994; Pellegrini-Giampietro et al., 1988) were previously reported. Adenylyl cyclase activity was determined using a camp radioimmunoassay kit in transfected cells or brain slices as described (Lombardi et al., 1993; Thomsen et al., 1992, 1993). The mGluR agonist-induced accumulation of labeled PET in hippocampal brain slices preloaded with [3H]glucocorticoid was used as a measure of PLD activity. Incubation of mGluR agonists and antagonists in the presence of 170 mM ethanol, organic extraction and thin layer chromatography separation of [3H]PET were performed as recently described in detail (Pellegrini-Giampietro et al., 1996).

Results

Effects of AIDA on phosphoinositide hydrolysis in mammalian cells transfected with mGluR1α or mGluR5α. AIDA reduced in a concentration-dependent manner the stimulation of phosphoinositide hydrolysis induced by L-glutamate in transfected BHK cells expressing either mGluR1α or mGluR5α (fig. 1). The IC50 value for this effect was 214 μM (95% confidence limits, 148–280) in mGluR1α cells and >1 mM in mGluR5α cells. Figure 1 shows that at 1 mM, AIDA antagonized the effects of 10 μM glutamate acting on mGluR1α by 90% but those of 5 μM glutamate on mGluR5α by only 32%. Glutamate was used at a higher concentration in mGluR1α cells because the cells are known
to exhibit lower sensitivity to agonists (Brabet et al., 1995, present work). In transfected cells expressing mGluR1a, the effects of AIDA against test concentrations (Brabet et al., 1995; Thomsen et al., 1993) of glutamate (10 μM) or (1S,3R)-ACPD (300 μM) gave a similar concentration-response curve (fig. 2). However, when [3H]IP formation was induced by a relatively large concentration of quisqualate (3 μM) in these cells, AIDA (1 mM) reduced PLC activity by only 50%.

AIDA (at 100, 300 and 500 μM) shifted to the right the concentration-response curve obtained by stimulating [3H]IP formation in cells expressing mGluR1a with (1S,3R)-ACPD (fig. 3). The maximal response to (1S,3R)-ACPD was not reduced at any concentration of AIDA, suggesting a competitive antagonism for this drug. A Schild plot was constructed with the (1S,3R)-ACPD curves obtained in the presence of 30, 100, 300 and 500 μM AIDA (data not shown): the slope of the Schild regression was not significantly different from 1. The calculated pA2 was 4.21 and was not significantly different from that reported for (S)-4C-PG (4.46), the most potent mGluR1a antagonist described so far (Hayashi et al., 1994; Watkins and Collingridge, 1994). When tested at the same concentrations in cells expressing mGluR5a (fig. 3), AIDA reduced the (1S,3R)-ACPD-induced formation of [3H]IP only at the highest concentration (500 μM).

In a separate set of experiments, AIDA (300 μM) was added to the incubation medium of cells expressing mGluR1a and maintained for 2 days. Cells were then extensively washed and tested for PLC activity with different concentrations of (1S,3R)-ACPD. Figure 4 shows that long-term exposure to the antagonist increased the cell response to each concentration of agonist, including the concentrations already giving a maximal response under control conditions.

Effects of AIDA on forskolin-activated cAMP formation in mammalian cells transfected with mGluR2 or mGluR4a. Glutamate and (1S,3R)-ACPD inhibit 10 μM forskolin-stimulated cAMP formation in BHK (fig. 5) and in other types of transfected cells expressing either mGluR2 or mGluR4a (Hayashi et al., 1994; Tanabe et al., 1993; Thomsen et al., 1994a). Similarly, the (carboxyphenyl)glycine (S)-4C-PG (10−500 μM) reduced forskolin-activated adenylate cyclase activity in mGluR2-expressing cells in a manner comparable to (1S,3R)-ACPD (fig. 5) (see also Hayashi et al., 1994; Watkins and Collingridge, 1994). The maximal degree of inhibition was ~60% and was achieved at 300 μM. AIDA (≤1 mM) did not modify the inhibitory action of a concentration of (1S,3R)-ACPD (300 μM) that gave a quasimaximal response in cells expressing mGluR2 (fig. 5) or in cells expressing mGluR4a. However, a modest agonist activity on mGluR2 was observed when 1 mM AIDA was used (fig 5). Because an exchange between AIDA and intracellular glutamate could account for these results (Thomsen et al., 1994b), experiments were performed to rule out this possibility. The negative results obtained indicate that AIDA (≤1 mM) does not interact with glutamate carriers in BHK cells (data not shown).

Effects of AIDA on PLC, adenylate cyclase or PLD activity in rat brain slices. Incubation of hippocampal slices with (1S,3R)-ACPD (30–300 μM) stimulated PLC activity in a concentration-dependent manner; at 100 μM, (1S,3R)-ACPD increased the formation of [3H]IPs by ~6-fold over basal values. Figure 6 shows that the latter effect was antagonized by AIDA (1–1000 μM) in a concentration-depen-
The particular shape of the inhibitory curve may be ascribable to the fact that at 1 mM AIDA interacts with at least two mGluR subtypes coupled to phosphoinositide hydrolysis (i.e., mGluR1 and mGluR5; see fig. 1). Other mGluR antagonists such as (S)-4C-PG and (+)-MCPG, which do not appear to interact with mGluR5 (Brabet et al., 1995), at 1 mM reduced the effect of 100 μM (1S,3R)-ACPD on [3H]IP formation by only 20% to 35% (fig. 6). AIDA was also tested on the inhibitory effect provided by 300 μM (1S,3R)-ACPD on the formation of cAMP induced by 30 μM forskolin in rat hippocampal and striatal slices (table 1). Even at high concentrations (500–1000 μM), AIDA failed to modify the effects of (1S,3R)-ACPD, whereas (+)-MCPG proved to be an efficacious antagonist. In line with the data obtained in mGluR2-transfected cells, a modest agonist activity on mGluRs negatively coupled to cAMP formation was observed in slices when large concentrations of AIDA (1000 μM) were tested (table 1).

Finally, the selectivity of AIDA for PLC-coupled mGluRs was further investigated by studying whether the drug was able to interact with presumably distinct mGluRs coupled to PLD (Boss et al., 1994; Pellegrini-Giampietro et al., 1996). As reported in figure 7, AIDA (300–1000 μM) did not stimulate PLD activity or inhibit the effects of a concentration of (1S,3R)-ACPD (100 μM) that induced a quasimaximal formation of [3H]PET in hippocampal slices. Conversely, (+)-MCPG displayed a complex profile in this system, possessing both agonist and antagonist activities (fig. 7).

Effects of AIDA on (1S,3R)-ACPD modulation of ν-[3H]aspartate output from rat cortical slices. (1S,3R)-ACPD (10–300 μM) significantly potentiated the depolarization-induced release of neurotransmitter in rat cerebro-cortical synaptosomes (Herrero et al., 1992) and cortical slices (Lombardi et al., 1994, 1996), as well as in the rat parietal cortex in vivo (Cozzi et al., 1996), with a pharmacological profile suggesting the involvement of group 1 mGluRs. In other brain areas such as the striatum, a similar concentration of (1S,3R)-ACPD inhibits the depolarization-induced output of ν-[3H]aspartate, probably because the agonist activates mGluRs of the second group in this area (Lombardi et al., 1993, 1994). When AIDA was added at 30 to 100 μM to the buffer solution perfusing cortical slices, the potentiating effects of 100 μM (1S,3R)-ACPD on KCl-induced ν-[3H]aspartate output were reduced (fig. 8). A larger concentration of AIDA (300 μM) perfused together with (1S,3R)-ACPD not only antagonized its potentiating effect but also reversed it into an inhibition of KCl-induced ν-[3H]aspartate outflow. A similar phenomenon was obtained with (S)-4C-PG, which prevented the potentiation of (1S,3R)-ACPD at 30 μM and caused a reduction of KCl-stimulated ν-[3H]aspartate output at 100 to 300 μM. On the contrary, at the higher concentra-
tion tested (500 μM), (+)-MCPG was able to completely inhibit the potentiation of release induced by (1S,3R)-ACPD without causing a reduction in the depolarization-induced output of d-[3H]aspartate (fig. 8).

Behavioral effects of i.c.v. injections of AIDA in mice. Mice injected i.c.v. with 5 μl of saline containing AIDA (0.01–10 nmol) and then placed in an open field exhibited some difficulty in the initiation of movement. However, mild stimuli, such as a light pinch on the tail, started an apparently normal ambulatory behavior that could not be distinguished from that of saline-injected controls. No obvious motor coordination deficit or ataxia was present. This apparent difficulty in initiating normal exploratory behavior lasted ~30 min. In other groups of animals, we then investigated the effects of AIDA (0.01–10 nmol i.c.v.) on the licking latency in the hot plate test and on the number of abdominal contractions after i.p. injection of 0.6% acetic acid. The results are reported in figure 9 and indicate that i.c.v. administration of AIDA caused mild analgesia, which at its maximum was comparable to that obtained with the systemic administration of 5 mg/kg morphine (O’Callaghan and Holtzman, 1976). Unexpectedly, the analgesic effect was no longer present when larger doses (0.1–10 μmol) of AIDA were used (data not shown).

Fig. 5. Effects of (1S,3R)-ACPD, AIDA and (S)-4C-PG on forskolin-activated cAMP formation in transfected BHK cells expressing mGluR2. Values are expressed as percentage of the formation of cAMP induced by 10 μM forskolin (basal levels of cAMP were 4.1 ± 0.6 pmol/mg of protein; forskolin increased these levels by ~10-fold). Values are mean ± S.E.M. of at least three experiments conducted in triplicate. ***P < .001 vs. forskolin alone.

Fig. 6. AIDA, (S)-4C-PG and (+)-MCPG reduce, in a concentration-dependent manner, the formation of [3H]IP induced by (1S,3R)-ACPD in rat hippocampal slices. Slices prelabeled with [3H]inositol were exposed to 100 μM (1S,3R)-ACPD in the presence of increasing concentrations of the antagonists for 15 min in the presence of 10 mM LiCl. The results are expressed as percentage of (1S,3R)-ACPD response minus basal levels of [3H]IP formation. Basal radioactivity found in the fraction corresponding to inositol phosphates was 15,900 ± 2,100 dpm/mg of protein; the exposure to 100 μM (1S,3R)-ACPD increased these levels by ~6-fold. Values are mean ± S.E.M. of at least five experiments conducted in triplicate.

Table 1
Effects of mGluR agents on forskolin-induced cAMP formation in rat hippocampal and striatal slices
Values are mean ± S.E.M. of at least 3 experiments conducted in triplicate.

<table>
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<tr>
<th></th>
<th>Hippocampus</th>
<th>Striatum</th>
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<tr>
<td></td>
<td>pmol/mg of protein</td>
<td>pmol/mg of protein</td>
</tr>
<tr>
<td>Control</td>
<td>31 ± 4</td>
<td>22 ± 5</td>
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<tr>
<td>Forskolin (30 μM)</td>
<td>441 ± 98a</td>
<td>318 ± 75a</td>
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<tr>
<td>Forskolin (30 μM) + (1S,3R)-ACPD (300 μM)</td>
<td>193 ± 11ab</td>
<td>231 ± 44abc</td>
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<tr>
<td>Forskolin (30 μM) + AIDA (500 μM)</td>
<td>380 ± 15a</td>
<td>280 ± 60a</td>
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<tr>
<td>Forskolin (30 μM) + AIDA (1000 μM)</td>
<td>200 ± 20abc</td>
<td>250 ± 30abc</td>
</tr>
<tr>
<td>Forskolin (30 μM) + (1S,3R)-ACPD (300 μM) + AIDA (500 μM)</td>
<td>171 ± 23abc</td>
<td>232 ± 24abc</td>
</tr>
<tr>
<td>Forskolin (30 μM) + (1S,3R)-ACPD (300 μM) + AIDA (1000 μM)</td>
<td>190 ± 15abc</td>
<td>223 ± 18abc</td>
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<tr>
<td>Forskolin (30 μM) + (1S,3R)-ACPD (300 μM) + (+)-MCPG (1000 μM)</td>
<td>425 ± 11a</td>
<td>341 ± 50a</td>
</tr>
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a P < .01 vs. control.
b P < .05 vs. 30 μM forskolin alone.
mGluR an-
response in cells expressing mGluR1a was considerably lower
mediated phosphoinositide hydrolysis (Pellicciari et al., 1995). In this study, AIDA prevents almost completely the
formed under (1S,3R)-ACPD-free (basal) conditions. Each column represents the mean ± S.E.M. of at least five experiments conducted in triplicate. "P < .01 vs. basal. "P < .01 vs. (1S,3R)-ACPD alone.

**Discussion**

Taken together, our results obtained in mammalian transfected cells and rat brain slices show that AIDA is a selective antagonist of group 1 mGluRs with preferential activity on mGluR1a over mGluR5a. The antagonist activity of AIDA on mGluR1a was competitive in nature and was better appreciated when L-glutamate or (1S,3R)-ACPD, rather than relatively elevated concentrations of quisqualate, was used as stimulating agents in transfected cells expressing mGluR1a. Similarly, in a recent study on the effects of (carboxyphenyl)glycine derivatives in transfected cells expressing mGluR1a or mGluR5a, it was reported that the apparent potency of mGluR antagonists depends on the agonist used to activate these receptors (Brabet et al., 1995). This phenomenon was particularly evident when weak antagonists were tested in the presence of potent agonists and may account for the diverse IC_{50} values that have been reported for mGluR antagonists. We previously reported (Pellicciari et al., 1995) that the IC_{50} value of AIDA inhibiting the L-glutamate response in cells expressing mGluR1a was considerably lower (7 μM) than that found in the present experiments (214 μM). However, the former data were obtained in a situation in which AIDA caused only a partial blockade on mGluR1a-mediated phosphoinositide hydrolysis (Pellicciari et al., 1995). In this study, AIDA prevents almost completely the stimulation of [3H]IP formation induced by (1S,3R)-ACPD, although it appears to be less potent.

In rat hippocampal slices, another model in which the antagonism of the formation of [3H]IP induced by (1S,3R)-ACPD was evaluated, the maximal inhibition (~70%) observed with 1 mM AIDA was significantly higher than that (~30%) obtained with the same concentrations of (+)-MCPG or (S)-4C-PG, two (carboxyphenyl)glycines known to interact with PLC-linked mGluRs (Brabet et al., 1995; Hayashi et al., 1994; Kingston et al., 1995). In addition, the concentration-response curve of AIDA had a biphasic shape, suggesting that in this region, relatively low concentrations of AIDA (≤0.1 mM) selectively block mGluR1, whereas larger concentrations (0.3–1 mM) also reduce the effect of (1S,3R)-ACPD on mGluR5. This hypothesis is in line with the observation that both mGluR1 and mGluR5 are expressed in rat hippocampus (Abe et al., 1992; Martin et al., 1992, Masu et al., 1991; Shigemoto et al., 1993).

When transfected BHK cells expressing mGluR1a were preexposed to AIDA for 48 hr, [3H]IP formation was significantly increased under basal conditions and after stimulatory concentrations of (1S,3R)-ACPD. This observation suggests that in transfected BHK cells, mGluR1a responses may be regulated by plastic changes of the system and that under basal conditions, receptors may be partially desensitized by the presence of significant concentrations of L-glutamate in the incubation medium. This hypothesis is in line with observations obtained using a Syrian hamster cell line transfected with mGluR1 plus a glutamate transporter to keep the concentrations of the excitatory amino acid low at the receptor level (Desai et al., 1995); when the transporter was coexpressed, [3H]IP formation induced by mGluR1 agonists was notably higher than that seen in its absence. Plastic changes of mGluR1 have also been described in primary cultures of cerebellar granular cells (Catania et al., 1991; Favaron et al., 1992), but the lack of appropriate antagonists has not yet allowed a detailed study of their basic mechanisms. Studies are currently in progress in our laboratory to elucidate whether the increased mGluR1a response in cells preexposed to AIDA could be due to an increased number of receptors, to changes in receptor affinity or to modifications in effector coupling.

In BHK cells transfected with mGluR2, AIDA had no effect on forskolin-induced stimulation of adenylyl cyclase activity of ≥300 μM but had a modest inhibitory activity at large concentrations (500–1000 μM). Similarly, (S)-4C-PG (30–300 μM) displayed agonist activity on mGluR2 by reducing forskolin-induced cAMP formation (see also Hayashi et al., 1994; Watkins and Collingridge, 1994). AIDA did not affect the inhibitory action of (1S,3R)-ACPD on forskolin-induced cAMP formation in rat striatal or hippocampal slices, but in line with the observations in transfected cells, it displayed agonist activity when large concentrations were used. In these preparations, (+)-MCPG antagonized the effects of (1S,3R)-ACPD, confirming its activity as an antagonist of mGluRs of the second group. In addition, AIDA was tested for its possible interaction with mGluRs coupled to PLD, which have been recently described in brain slices (Boss and Conn, 1992; Holler et al., 1993), and appear to have a pharmacological profile that is distinct from that of any known mGluR subtype linked to PLC or adenylyl cyclase (Pellegrini-Giampietro et al., 1996). (+)-MCPG acts as an agonist/antagonist on PLD-coupled mGluRs, whereas AIDA (≤1 mM) neither stimulates PLD activity nor modifies the effect of the agonist (1S,3R)-ACPD.
mGluRs is the potentiation of depolarization-induced release of transmitter in cortical preparations (Coffey et al., 1994; Lombardi et al., 1994, 1996). On the contrary, activation of mGluRs negatively linked to adenylate cyclase inhibits release in other brain preparations and, in particular, in striatal slices (Anwyl, 1991; Lombardi et al., 1993, 1994; Lovinger, 1991). (+)-MCPG prevents both the stimulatory and the inhibitory effects of (1S,3R)-ACPD on transmitter release (Lombardi et al., 1993), most likely because it is an antagonist acting on mGluRs of both the first and second group. The results reported in this work for other mGluR antagonists on (1S,3R)-ACPD modulation of D-[^3H]aspartate output from rat cortical slices are intriguing. When (S)-4C-PG was studied, we observed that increasing concentrations reverted the antagonism of the potentiating effect of (1S,3R)-ACPD into a potent inhibition of KCl-induced output. It is reasonable to propose that the antagonism is mediated by blockade of mGluR1, whereas the inhibition is due to the simultaneous agonist activity of (S)-4C-PG and (1S,3R)-ACPD on mGluRs of the second group negatively linked to cAMP formation, such as mGluR2 (see fig. 5). AIDA also antagonized the positive effect of (1S,3R)-ACPD at lower doses and reverted it into an inhibition of KCl-induced output, although only at the higher concentration tested (1 mM). In this case, because the compound is inactive on mGluR2, 1 mM AIDA could be blocking mGluR1 and mGlu5, allowing the interaction between (1S,3R)-ACPD and mGluRs of the second group. The use of different antagonists at specific concentrations (e.g., 100 μM in fig. 8) may thus reveal the opposite effects of (1S,3R)-ACPD on transmitter release. These data could perhaps be helpful for the interpretation of otherwise contradictory results regarding the effects of mGluR agonists and antagonists on mechanisms leading to long-term potentiation or other forms of synaptic plasticity (Bashir et al., 1993; Brown et al., 1994; Chinestra et al., 1994; O’Connor et al., 1994).

Finally, we injected AIDA i.c.v. in mice and evaluated their behavior. When treated animals were placed in an open field, we expected to observe the symptomatology described in detail for mice lacking mGluR1: wide base standing position, tremor, ataxia and loss of the righting reflex (Aiba et al., 1994b; Conquet et al., 1994). No dose of AIDA, however, elicited such effects, the righting reflex was always present, and the ambulatory behavior of treated animals was apparently identical to that of control mice. A careful observation of i.c.v. injected mice, however, revealed that they had a ten-
ency to stay immobile in the center of the open field, possibly because they had difficulties in the initiation of movements. A mild stimulation would interrupt this immobility, and the animals would start to explore the new environment in a manner comparable to those injected with saline. It is possible that AIDA injected i.c.v. might not reach the cerebellar cortex in sufficient concentration; this could explain the discrepancies between our study and those using mGluR1 knock-out animals. However, it is also possible that mice lacking mGluR1 display motor deficit in adulthood because the receptor is required to learn specific motor skills during development. Obviously, animals treated with receptor antagonists in adult life will not exhibit motor impairment because they have mastered motor skills at a time when mGluR1 was not blocked.

It has been shown that antagonists of mGluRs of the first group reduce electrophysiological responses evoked by nociceptive stimuli in the spinal cord and in the thalamus (Salt and Eaton, 1994; Young et al., 1994). We thus tested the effects of AIDA injected i.c.v. in two models widely used to uncover drug actions on the nociceptive reflex: the hot plate test and the writhing test. AIDA delayed the pain reaction (paw licking) of mice placed on a hot plate at 52°C and significantly reduced the number of writhes observed after i.p. administration of diluted acetic acid. The analgesic effect of AIDA was comparable to that of 5 mg/kg s.c. morphine. However, it should be noted that AIDA-mediated antinociception had a bell-shaped dose-response curve because it was no longer present when large doses (0.1–10 μmol) of the compound were administered. This could suggest that mGluR1 and mGluR5 have opposite effects on the antinociceptive reflex, although the present experiments do not rule out alternative hypotheses. More potent and selective mGluR antagonists are required to permit a satisfactory explanation for these observations.

In conclusion, AIDA is an antagonist of mGluRs coupled to PLC activity with potency for mGluR1a superior to that for mGluR5a. When used in transfected cells or in brain slices, it does not affect mGluRs of the second or third group at a concentration of ≤1 mM. Thus, AIDA appears to be one of the most potent and selective mGluR1 antagonist described so far. In addition, AIDA antagonizes the potentiation of (1S,3R)-ACPD on the depolarization-induced output of transmitter from cortical slices, and when injected i.c.v., it has mild analgesic effects.

References


